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(54) Formation of niosomes dispersed in an aqueous phase

(57) A method for forming niosomes, formed from a non-ionic lipid phase and an aqueous phase, the niosomes comprising a single lipid lamella, or two or more substantially concentric lipid lamellae, encapsulating an aqueous phase E, the niosomes being dispersed in an aqueous medium D, wherein, before the niosomes are formed, at least one cholesterol phosphate, in the form of its free acid or neutralized with an ammonium, alkali metal or alkaline earth metal cation, is added to the non-ionic lipid(s) intended to form the niosome lamella (e) in an amount of from 1 to 40% by weight relative to the total weight of the lipid phase. Cosmetic creams are suitably prepared by the process.

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SPECIFICATION

Method for facilitating the formation of niosomes dispersed in an aqueous phase and for improving the stability and the degree of encapsulation thereof and corresponding dispersions

The present invention relates to a method for preparing aqueous dispersions of niosomes, these dispersions being suitable for use, for example, in the cosmetic, pharmaceutical and foodstuff fields. The invention especially relates to a method for facilitating the formation, and improving the stability and degree of encapsulation, of niosomes in aqueous dispersions.

It is known that some lipids can form in the presence of water, a mesomorphic phase, which is a phase between the crystalline state and the liquid state. Some lipids which form mesomorphic phases may swell in an aqueous solution to form spherules dispersed in the aqueous medium, these spherules being made of multi-molecular layers, preferably bi-molecular layers.

Dispersions of lipid spherules have already been described in French Patent No.2,315,991; these spherules have a lamellar structure consisting of two or more lipid layers separated from one another by aqueous phase layers. They can be used to encapsulate, within the aqueous compartments contained between the lipid layers, water-soluble active substances, for example pharmaceutical or cosmetic active substances to protect them from external conditions. The lipids which can be used to form the spherules may be ionic compounds, in which case liposomes are obtained, of non-ionic compounds, in which case niosomes are obtained.

The present invention is concerned with noisomes.

When niosomes are prepared, various additives may be combined with the non-ionic lipid compounds in order to modify the permeability of the surface charge of the spherules. A number of additives for this purpose are mentioned in French Patents 2,315,991, 2,485,921 and 2,490,504. Thus, it is known that, when it is desired to reduce the permeability of the vesicles, sterols, especially cholesterol may be added to the lipid compounds to increase the rigidity of the multilayers.

It is also known that the incorporation of electrically charged molecules into the walls of niosomes affects the properties of these multilayers. Charged lipids, such as dicetyl phosphate, phosphatidic acid and long hydrocarbon chain quaternary ammonium compounds or amines improve the stability of the vesicles by preventing their flocculation and, therefore, their fusion, even in the presence of electrolytes, and make it possible to increase the degree of encapsulation of water-soluble substances by increasing the thickness of the aqueous lamellae which separate the lipid multilayers.

In the case of liposomes, it has been demonstrated by A. Colombat et al, Biochimie (1981), *63*, 795-798, that cholesterol phosphate, i.e. a hydrophilic ester of cholesterol, on the one hand provides the effects of a charged amphiphilic substance, that is increases the stability of liposomes and their degree of encapsulation, and, on the other hand, provides the effect of cholesterol, that is decreases the permeability of liposomes. However, it is observed that the introduction of more than 5% by weight of charged lipids into the vesicle membrane results either in a high permeability to solutes, or in recrystallization of the charged lipid. Thus although it is known that the lipid lamellae of liposomes can contain cholesterol phosphate, it is also known that this addition involves several disadvantages.

We have surprisingly discovered that cholesterol phosphates do not have the disadvantages mentioned above when they are combined with niosomes, and that they are different from other charged lipids in that they can be introduced into the lipid membrane in an amount up to 40% by weight without recrystallization. In relatively high percentages in the membrane (10% by weight), they only give rise to a low permeability.

The present invention therefore provides a method for forming, and improving the degree of encapsulation and stability of niosomes, which comprises forming niosomes, formed from a non-ionic lipid phase and an aqueous phase, the niosomes comprising a single lipid lamella, or two or more substantially concentric lipid lamellae, encapsulating an aqueous phase E, the niosomes being dispersed in an aqueous medium D, wherein, before the niosomes are formed, at least one cholesterol phosphate, in the form of its free acid or neutralized with an ammonium, alkali metal or alkaline earth metal cation, is added to the non-ionic lipid(s) intended to form the niosome lamella(e) in an amount of from 1 to 40% by weight relative to the total weight of the lipid phase.

In practice, the maximum amount of cholesterol phosphate usable depends on the nature of the lipid used; it may be, for example, from 10 to 40% by weight relative to the weight of the lipid phase of the niosomes.

A substituted or unsubstituted ammonium, sodium or potassium acid cholesterol phosphate is preferably used.

Any of the previously known and described methods may be used for carrying out the dispersion of the niosomes in the aqueous phase D.

It is possible to use, for example, a method wherein the lipids are dissolved in a volatile solvent, and a thin lipid film is formed, for example on the sides of a jar, by evaporating the solvent. An aqueous phase E to be encapsulated is then introduced into the jar and the mixture is stirred, generally mechanically, until a dispersion of niosomes of the desired size is obtained. In this case, the aqueous phases D and E are necessarily identical.

It is also possible to use the method described in French Patent No.2,315,991, wherein the aqueous phase E is introduced into the liquid non-ionic lipid to form a plane lamellar phase, preferably at a temperature slightly above the melting point of the lipid, an aqueous phase, which may or may not be identical to the aqueous

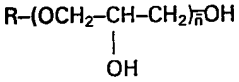
phase E, is added to the lamellar phase obtained, and the mixture is stirred vigorously, for example mechanically, to provide a dispersion of niosomes encapsulating the aqueous phase E in th aqueous phase D. Depending on the means employed for carrying out the dispersion (for example by an ultradispersing device, homogenizer and/or ultrasonic dispersing device) and the period of stirring (from 15 minutes to a few hours), niosomes with mean diameter varying from 0.025 to 5 microns may be obtained.

The method mentioned above is particularly well-suited to provide multilamellar niosomes. In the case where unilamellar niosomes are desired, the method described in French Patent 2,543,018 may be used for their preparation. According to this method, the lipid for forming the niosome lamella is solubilized in at least one water-insoluble solvent to provide a lipid solution. The lipid solution in the liquid state, in a container, is then subjected to a pressure p_1 and a temperature Θ_1 and the aqueous phase E is subjected to a pressure p_2 and a temperature Θ_2. The lipid solution is injected into the aqueous phase so that the solvent of the lipid solution evaporates when it comes into contact with the aqueous phase. The injection is carried out at a low rate so as to initially form droplets, and the pressure p_2 is lower than pressure p_1 and lower than the vapor pressure of the solvent of the droplets at temperature Θ_2.

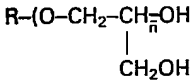
The cholesterol phosphate may be added at any time before the niosomes are formed, for example while passing through the formation of a lamellar phase, or either before or after the preparation of the said lamellar phase.

The lipids used for the preparation of the spherules are preferably non-ionic amphiphilic substances of natural or synthetic origin, containing one or more saturated or unsaturated, straight or branched, long chain hydrocarbon groups containing, in particular, 8 to 30 carbon atoms, such as oleyl, lanolyl, tetradecyl, hexadecyl, isostearyl, lauryl or alkylphenyl groups, and one or more hydrophilic groups per molecule.

For these non ionic amphiphilic substances, it is preferred that the hydrophilic groups are polyoxyethylenated or polyglycerolated groups, or groups derived from esters of polyhydric alcohols, which can optionally be oxyethylenated, or hydroxyamide derivatives. These non-ionic lipid compounds are advantageously: a straight or branched-chain polyglycerol ether of formula:



or



wherein $\bar{n}$ has a mean statistical value of from 1 to 6 and R is a straight or branched-chain, saturated or unsaturated, aliphatic group containing 12 to 30 carbon atoms, a hydrocarbon group of a lanolin alcohol or a 2-hydroxyalkyl residue of a long chain α-diol;

a straight or branched chain polyglycerol ether containing two fatty chains;

a polyoxyethylenated fatty alcohol;

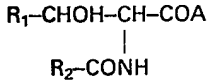
an ether of a polyhydric alcohol;

a polyoxyethylenated sterol;

an ester of a polyhydric alcohol which may optionally be oxyethylenated, in particular, a polyoxyethylenated sorbitol ester; or

a glycolipid of natural or synthetic origin, for example a cerebroside; or

a hydroxyamide such as those described in FR-A-2,588,256 of formula:

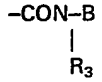


wherein:

R_1 is a C_7–C_{21} alkyl or alkenyl group;

R_2 is a saturated or unsaturated C_7–C_{31} hydrocarbon group; and

COA is either a group of formula:



wherein B is a group derived from a mono- or polyhydr xylated primary r secondary amine and R_3 is hydrogen or a methyl, ethyl or hydroxyethyl group; or

–COOZ

wherein Z is the residue of a C_3–C_7 polyhydric alcohol.

Various additives may be combined, in a known manner, with the lipid in order to modify the permeability or the surface charge of the spherules. Examples of such additives are long chain diols and alcohols, sterols, for example cholesterol and β-sitosterol, long chain amines, hydroxyalkyl amines, polyoxyethylenated fatty amines, long chain amino alcohol esters and their salts, phosphoric acid esters of fatty alcohols, for example sodium dicetyl phosphate and alkyl sulfates, for example sodium cetyl sulfate, and ionic derivatives of sterols other than cholesterol phosphates.

In order to form the niosome dispersion, 0.5 to 25% by weight of the lipid, or non-ionic amphiphilic substance, relative to the total weight of the niosome dispersion obtained, may advantageously be used.

The walls of the spherules may, for example, contain at least one lipid-soluble substance, for example a keratolytic agent such as retinoic acid, an anti-inflammatory agent such as β-methasone-17-valerate or an antioxidant such as vitamin E, or an acetate thereof, or ascorbyl palmitate, which is especially useful when local application of the niosomes is envisaged.

The aqueous phase E may, for example, also be an aqueous solution of an active substance, and is preferably isoosmotic to the dispersion phase D. Phases D and E may be identical

The niosomes may be used in a cosmetic composition. In this case, the aqueous phase E encapsulated in the niosomes may, for example, comprise at least one of a moisturizer such as glycerine, sorbitol, pentaerythritol, inositol, pyrrolidonecarboxylic acid or a salt thereof; artificial tanning agent such as dihydroxyacetone, erythrulose, glyceraldehyde or γ-dialdehyde such as tartaraldehyde, combined with a colorant if required; a water-soluble sunscreen agent; an anti-perspirant; a deodorant; an astringent; a freshener, tonic, cicatrizing agent, keratolytic agent or depilatory; an animal or vegetable tissue extract; a perfumed water; a water-soluble colorant; an anti-dandruff agent; an anti-seborrheic agent; an oxidizing agent such as hydrogen peroxide or a reducing agent such as thioglycolic acid or a salt thereof.

The niosomes may also be used in a pharmaceutical composition. In this case the aqueous phase E encapsulated in the niosomes preferably comprises at least one of a vitamin, hormone, enzyme such as superoxide dismutase, vaccine, anti-inflammatory agent such as hydrocortisone, antibiotic or bactericide.

The aqueous phase D surrounding the niosomes may also, for example, comprise at least one water-immiscible liquid phase dispersed therein. This water-immiscible liquid phase is desirably an oil or a hydrocarbon, halogenated hydrocarbon, polysiloxane, or organic acid ester, ether or polyether. The quantity of the water-immiscible liquid phase dispersed in the aqueous phase D is advantageously from 2 to 70% by weight, relative to the total weight of the composition and the relative proportion by weight of the lipid forming the niosomes relative to the dispersed water-immiscible liquid phase is desirably from 0.02:1 to 10:1.

The oil used as a water-immiscible liquid is advantageously an ester of a fatty acid or polyhydric alcohol, especially a liquid triglyceride or an ester of a fatty acid and a branched alcohol of formula R_4–$COOR_5$, wherein R_4 is a residue of a higher fatty acid containing 7 to 19 carbon atoms and R_5 is a branched hydrocarbon chain containing 3 to 20 carbon atoms. If the oil is an ester of a fatty acid and a polyhydric alcohol, it is preferred that it is derived from sunflower, corn, soy bean, marrow seed, grape seed, sesame, sweet almond or jojoba oil and glycerol tricaprocaprylate. If the oil is an ester of a higher fatty acid and a branched alcohol, it is preferred that the oil is Purcellin oil.

Hexadecane, paraffin oil, perhydrosqualene, perfluorotributylamine, perfluorodecahydronaphthalene and a volatile silicone fluid may also advantageously be the water-immiscible liquid phase.

The aqueous phase D, which surrounds the niosomes, may for example, contain at least one adjuvant such as an opaquing agent, gelling agent, aroma, perfume, sunscreen or colorant. It is possible for those adjuvants which are lipid soluble to be dissolved in a water-immiscible liquid phase dispersed in the aqueous phase D if such a dispersion is used.

If it is desired that the dispersed water-immiscible liquid added to the continuous aqueous phase which surrounds the niosomes contains dissolved adjuvants, these adjuvants may, for example, be dissolved before carrying out the dispersion.

Examples of such adjuvants are sunscreens such as 2-ethylhexyl para-dimethylaminobenzoate or substances intended for improving the condition of dry or senile skin, in particular unsaponifiable compounds such as unsaponifiables derived from soya bean or avocado, tocopherols, vitamin E or F or antioxidants.

The dispersion of oil in water which may form the external medium for the dispersion of the niosomes may for example contain at least one additive, especially a gelling agent or a perfume. The additive is desirably added to the dispersion at the same time as the oil. The gelling agent may be introduced in an amount of from 0.1 to 2% by weight relative to the total weight of the composition. Examples of gelling agents are cellulose derivatives, algal derivatives, crosslinked polyacrylic acids and natural gums. Preferred gelling agents are hydroxyethylcellulose, a crosslinked polyacrylic acid sold by GOODRICH under the name "CARBOPOL 940" (Trade Mark), satiagum or tragacanth gum.

When a composition containing a dispersion of water-immiscible liquid is prepared, it is observed that the dispersion is stable without using any emulsifier.

The present invention also provides a dispersion of niosomes comprising a lipid lamella, r tw or more substantially concentric lipid lamellae, encapsulating an aqueous phase E, the niosomes being dispersed in an aqueous medium D, obtained by a method as herein defined.

The dispersion may contain spherules of several types in the aqueous medium D. In this case, spherules of each type may be prepared separately in different dispersions and the dispersions then mixed. A dispersion of liposomes of any known type may, for example, be added to the dispersion of niosomes produced by the process of the present invention.

Some examples of preparation implementing the invention and some examples of formulations illustrating the use of the spherule dispersion according to the invention are given below.

The preparation of cosmetic or pharmaceutical formulae given in the examples below is carried out in one or two stages.

In a first stage, an aqueous dispersion is prepared according to the method described in French Patent 2,315,991 (examples 1 to 3). The aqueous dispersion of lipid spherules is prepared from:

a non-ionic amphiphilic lipid,

cholesterol phosphate, used alone or in combination with cholesterol, and

cosmetic active substances of the lipid soluble and/or water-soluble type and demineralized water.

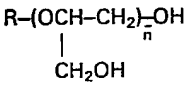
In a second optional stage, depending on the cosmetic or pharmaceutical nature of the formulation, oil may be added to the external medium, so as to form an oil-in-water system according to the method described in French Patents Nos. 2,485,921 and 2,532,191. Different cosmetic additives may also be added.

Example 1: Cream for the tratment of dry skin

1st phase:

The following products are weighed into a stainless steel beaker:

non-ionic amphiphilic lipid of formula



in which R is a hexydecyl radical

and n has a statistical mean value equal to 3	4.00 g
cholesterol	2.00 g

These two products are mixed by melting at a temperature of 110°C under a nitrogen atmosphere. The temperature of the molten mixture is then adjusted to 90°C. 20 g of demineralized water are added and the mixture obtained is homogenzied at a temperature of 70°C, using an ultradispersing device of the "Virtis" type until the average size of vesicles obtained is 0.5 micron.

2 g of cholesterol phosphate (acid form) are added at the same temperature and the mixture is homogenized until the uncombined lipid crystals disappear completely as monitored by examining under an optical microscope using polarized light.

The following are then added:

methyl parahydroxybenzoate (stabilizer)	0.30 g
glycerine	5.00 g
demineralized water	25.00 g

The mixture is homogenzied at a temperature of 70°C, using an ultradispersing device of the "Virtis" type until the average size of vesicles obtained is 0.5 micron.

2nd phase:

The following products are added to the above mixture:

almond oil	5.00 g
Cetiol LC, marketed by HENKEL (mixture of esters of C_8C_{10} acids and C_{12}–C_{18} fatty alcohols)	10.00 g

The whole mass is subjected to the action of a "Virtis" ultradispersing device until the oil globules have an average diameter of approximately 1 micron.

The following additives are finally added:

Perfume	0.40 g
Crosslinked polyacrylic acid sold by GOODRICH under the name "CARBOPOL 940"	0.40 g
Triethanolamine	0.40 g
Demineralized water	25.00 g

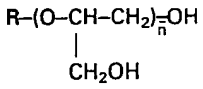
It should be noted that this composition remains stable for a period of more than 2 years.

This cream, applied locally once a day in subjects with dry skin, gives satisfactory results after 20 days of application.

Example 2: Concentrate for irritated skin

The following products are weighed into a stainless steel beaker:

non-ionic amphiphilic lipid of formula



in which R is a hexadecyl radical and
n has a statistical mean value equal to 3 7.60 g
cholesterol 7.60 g

These two products are mixed by melting at a temperature of 110°C under a nitrogen atomosphere, and the temperature of the molten mixture is then adjusted to 90°C.

40 g of demineralized water and 5 g of glycerine are added. The mixture obtained is homogenized at a temperature of 90°C and 0.8 g of cholesterol phosphate (acid form) is added.

The mixture is homogenized until the uncombined lipid crystals disappear completely, as monitored by examining under an optical microscope with polarized light.

The following products are then added:

methyl para-hydroxbenzoate (stabilizer) 0.30 g
demineralized water 38.70 g

The mixture is homogenized at a temperature of 70°C using an ultradispersing device of the "Virtis" type until the average size of vesicles obtained is approximately 0.3 micron.

It should be noted that this composition remains stable for a period of more than two years.

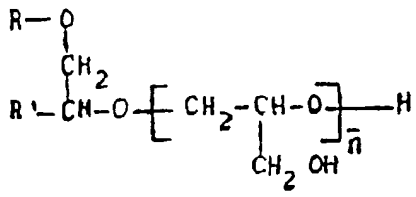
This cream, used for local application twice a day in subjects suffering from irritated skin affected by acne, reduces the irritation after one or two weeks of application.

Example 3: Milk for the care of irritated skin

1st preparation phase:

The following products are weighed into a stainless steel beaker:

Nonionic amphiphilic lipid of formula:



in which R is a dodecyl radical;
R' is an equimolar mixture of tetradecyl and hexadecyl radicals; and
ñ has a statistical mean value equal to 5.5 as determined by nuclear magnetic resonance 3.8 g
cholesterol phosphate (acid form) 0.2 g

The mixing of these two products is carried out by melting at 90°C. 10 g of demineralized water are added and the mixture obtained is homogenized at 90°C.

The following products are then added:

Methyl parahydroxybenzoate (stabilizer) 0.30 g
Glycerine 5.00 g
Demineralized water 50.70 g

The mixture is homogenized at 40°C using an ultradispersing device of the "Virtis" type until the average size of the spherules obtained is 0.2 micron.

2nd preparation phase:

15 g of sesame oil are added to the aqueous dispersion obtained above. The whole mixture is subjected to the action of a "Virtis" ultradispersing device until the oil globules are of a mean diameter of approximately 1 micron.

The following substances are finally added:

Perfume 0.40 g
Crosslinked polyacrylic acid sold by GOODRICH under the tradename "CARBOPOL 940" 0.40 g
Triethanolamine 0.40 g
Demineralized water 13.8 g

This milk, applied locally twice a day on subjects with an irritated skin, decreas s the irritati n after tw weeks of application.

Example 4: Milk for the treatment of dry skin

1st preparation phase:

The following products are weighed into a stainless steel beak r:

Nonionic amphiphilic lipid used in Example 3	4.8 g
Cholesterol phosphate (acid form)	3.2 g

The mixing of these two products is carried out by melting at 95°C. 20 g of demineralized water are added. The mixture obtained is homogenized at 90°C.

The following products are then added:

Methyl parahydroxybenzoate (stabilizer)	0.3 g
Glycerine	5.0 g
Demineralized water	36.7 g

The mixture is homogenized at 40°C using an ultradispersing device of the "Virtis" type until the mean size of spherules obtained is 0.2 micron.

2nd preparation phase:

15 g of sesame oil are added to the aqueous dispersion obtained above. The whole mixture is subjected to the action of a "Virtis" ultradispersing device until the oil globules are of a mean diameter of approximately 1 micron.

The following substances are finally added:

Perfume	0.40 g
Crosslinked polyacrylic acid sold by GOODRICH under the trade name "CARBOPOL 940"	0.40 g
Triethanolamine	0.40 g
Demineralized water	13.80 g

This milk, applied locally once a day on subjects with a dry skin, enables a substantial improvement in the state of the skin to be obtained after two weeks of application.

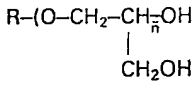
Example 5: Cream for the case of skin affected by acne

The entire preparation of this cream was carried out under yellow light from a sodium vapor lamp.

1st preparation phase:

The following products are dissolved in 200 ml of a solvent mixture (chloroform:methanol in a ratio of 1:1), in a 1 litre round-bottomed flask:

Nonionic lipid of formula:



in which R is a hexadecyl radical and ñ has a statistical mean value equal to 3

	3.8 g
Cholesterol	3.8 g
Cholesterol phosphate (acid form)	0.4 g
Retinoic acid marketed by ROCHE under the trade name "TRETINOINE"	0.025 g

The solvent is evaporated off using a rotary evaporator and the final traces of the solvent are removed using a vane pump for 1 hour.

The combination of lipids obtained are brought into contact with 20 g of demineralized water mixed with 3 g of glycerine. The mixture obtained is homogenized at 80°C.

0.3 g of methyl parahydroxybenzoate (stabilizer) dissolved in 38.675 g of demineralized water is then added.

The mixture is homogenized at 60°C using a "Virtis" ultradispersing device until the mean size of the spherules obtained is approximately 0.3 micron.

2nd preparation phase:

15 g of glycerol tricaprocaprylate are then added. The whole mixture is subjected to the action of a "Virtis" ultradispersing device so that the external phase of the oil dispersion c ntains globules of a mean diamet r f approximately 1 micron.

The following substances are finally added:

Perfume	0.4 g
Crosslinked polyacrylic acid sold by GOODRICH under the trade name "CARBOPOL 940"	0.4 g
Triethanolamine	0.4 g
Demineralized water	13.8 g

This cream, applied locally once or twice a day on subjects with acne-affected skin, enables a substantial

improvement to be observed after two weeks of application

Example 6: Vesicular preparation of corticoids

The following products are weighed into a stainless steel beaker:

Nonionic amphiphilic lipid used in Example 3	7.6 g
Cholesterol phosphate (acid form)	0.4 g
β-methasone-17-valerate (product marketed by LARKS)	0.08 g

The mixing of these products is carried out by melting at 90°C. 20 g of demineralized water are added. The mixture obtained is homogenized at 90°C.

The following products are then added:

Methyl parahydroxybenzoate (stabilizer)	0.3 g
Glycerine	5.0 g
Demineralized water	52.02 g

The mixture is homogenized at 40°C, using an ultradispersing device of the "Virtis" type until the mean size of the vesicles obtained is 0.2 micron.

The following products are finally added:

Crosslinked polyacrylic acid sold by GOODRICH under the trade name "CARBOPOL 940"	0.4 g
Triethanolamine	0.4 g
Demineralized water	13.8 g

This preparation, applied locally twice a day, on subjects suffering from dermatitis, enables a substantial improvement to be observed after a few days of application.

Example 7: Aqueous dispersion of lipid vesicles

The following products are dissolved in 200 ml of solvent (chloroform:methanol in a ratio of 1:1), in a 1 litre round-bottomed flask:

Nonionic amphiphilic lipid used in Example 3	7.6 g
Cholesterol phsophate (acid form)	0.4 g
α-tocopherol acetate (product marketed by ROCHE)	0.2 g
α-tocopherol (product marketed by ROCHE)	0.2 g
Ascorbyl palmitate (product marketed by ROCHE)	0.4 g

The solvent is evaporated off using a rotary evaporator and the final traces of the solvent are removed using a vane pump for 1 hour.

The combination of lipids obtained is brought into contact with 20 g of demineralized water and the mixture obtained is homogenized at 90°C.

The following products are then added:

Methyl parahydroxybenzoate (stabilizer)	0.3 g
Glycerine	5.0 g
Demineralized water	51.3 g

The mixture is homogenized at 40°C using a "Virtis" ultradispersing device until the mean size of the vesicles obtained is 2.0 micron.

The following products are finally added:

Crosslinked polyacrylic acid sold by GOODRICH under the trade name "CARBOPOL 940"	0.4 g
Triethanolamine	0.4 g
Demineralized water	13.8 g

This dispersion, applied locally once a day, on subjects with certain symptoms of ageing, gives satisfactory results after four weeks of application.

CLAIMS

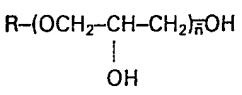
1. A method for forming niosomes, formed from a non-ionic lipid phase and an aque us phase, th niosomes comprising a single lipid lamella, or two or more substantially concentric lipid lamellae, encapsulating an aqueous phase E, the niosomes being dispersed in an aqueous medium D, wherein, befor the niosomes are formed, at least one cholesterol phosphate, in the form of its free acid or neutralized with an ammonium, alkali metal or alkaline earth metal cation, is added to the non-ionic lipid(s) intended to form the niosome lamella(e) in an amount of from 1 to 40% by weight relative to the total weight of the lipid phase.

2. A method according to claim 1 wherein a cholesterol phosphat in its free acid form r in the form of a substituted or unsubstituted ammonium, sodium or potassium salt is employed.

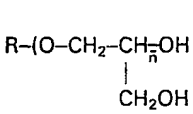
3. A method according to claim 1 or 2 wherein the aqueous phase E is introduced into a liquid non-ionic lipid to form a plane lamella phase, an aqueous phase is added and the mixture is stirred vigorously to provide a dispersion of niosomes in the aqueous medium D.

4. A method according to any one of claims 1 to 3, wherein the lipid is at least one non-ionic amphiphilic substance of natural or synthetic origin, containing one or more long chain hydrocarbon groups and one or more hydrophilic groups per molecule.

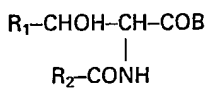
5. A method according to claim 4 wherein the non-ionic amphiphilic substance is:
a straight or branched-chain polyglycerol ether of formula:



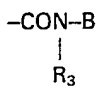
or



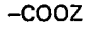
wherein $\bar{n}$ has a statistical mean value of from 1 to 6 and R is a straight or branched-chain, saturated or unsaturated, aliphatic group containing 12 to 30 carbon atoms, a hydrocarbon group of a lanolin alcohol or a 2-hydroxyalkyl residue of a long-chain α-diol;
a straight or branched chain polyglycerol ether containing two fatty chains;
a polyoxyethylenated fatty alcohol;
a polyoxyethylenated sterol;
an ether of a polyhydric alcohol;
an ester of a polyhydric alcohol which may optionally be oxyethylenated; or
a glycolipid of natural or synthetic origin; or
a hydroxyamide of formula:



wherein:
R_1 is a C_7–C_{21} alkyl or alkenyl group;
R_2 is a saturated or unsaturated C_7–C_{31} hydrocarbon group; and
COA is either a group of formula:



wherein B is a group derived from a mono- or polyhydroxylated primary or secondary amine and R_3 is hydrogen or a methyl, ethyl or hydroxyethyl group; or



wherein z is a residue of a C_3–C_7 polyhydric alcohol.

6. A method according to any one of claims 1 to 5 wherein at least one additive is added to the lipid phase, the additive being a long chain diol or alcohol, a sterol, a long chain amine, a hydroxyalkylamine, a polyoxyethylenated fatty amine, a long chain amino alcohol ester or a salt thereof, a phosphoric acid ester of a fatty alcohol, an alkyl sulfate or an ionic derivative of a sterol other than cholesterol phosphate.

7. A method according to any one of claims 1 to 6 wherein the lipid phase is present in an amount of from 0.5 to 25% by weight relative to the total weight of the niosome dispersion obtained.

8. A method according to any one of claims 1 to 7 wherein at least one lipid-soluble substance is added to the lipid phase intended to form the spherules.

9. A method according to claim 8 wherein the lipid-soluble substance is a keratolytic agent, anti-inflammatory agent or antioxidant.

10. A method according to any one of claims 1 to 9 wherein the aqueous phase E is an aqueous solution of at least one active substance.

11. A method according to claim 10 wherein the aqueous phase E is isoosmotic to the phase D.

12. A method according to claim 10 or 11 wherein the aqueous phases D and E are identical.

13. A method according to any one of claims 10 to 12 wherein the aqueous phase E comprises at least one of a moisturizer, artificial tanning agent, water-soluble sunscreen, anti-perspirant, deodorant, astringent,

freshening product, tonic product, cicatrizing product, keratolytic pr duct, depilat ry product, perfumed water, water-soluble colorant, anti-dandruff agent, anti-seborrhic agent, oxidizing agent, r ducing agent or animal or vegetable tissue extract and the niosomes produced ar suitable for use in a cosmetic composition.

14. A method according to any one of claims 10 to 12 wherein the aqueous phase E comprises at least one of a vitamin, hormone, enzyme, vaccine, anti-inflammatory agent, antibiotic or bactericide and the niosomes produced are suitable for use in pharmaceutical preparations.

15. A method according to any one of claims 1 to 14 wherein the niosomes dispersed in the aqueous medium D are mixed with at least one water-immiscible liquid phase L to form at least one water-immiscible liquid phase dispersed in the aqueous phase D.

16. A method according to claim 15 wherein from 2 to 70% by weight, relative to the total weight of the composition, of the water-immiscible liquid phase L is used, the proportion by weight of the lipid forming the niosomes relative to the dispersed water-immiscible liquid phase being from 0.02:1 to 10:1.

17. A method according to claim 15 or 16 wherein the water-immiscible liquid phase L is an oil, hydrocarbon, halogenated hydrocarbon, polysiloxane, organic acid ester, ether or polyether.

18. A method according to claim 17 wherein the water-immiscible liquid phase L is an ester of a fatty acid and a polyhydric alcohol, an ester of a fatty acid and a branched alcohol of formula R_4–$COOR_5$, wherein R_4 ia residue of a higher fatty acid containing from 7 to 19 carbon atoms and R_5 is a branched hydrocarbon chain containing from 3 to 20 carbon atoms, hexadecane, a paraffin oil, perhydrosqualene, perfluorodecahydronaphthalene or perfluorotributylamine.

19. A method according to any one of claims 1 to 18, wherein the aqueous phase D comprises at least one opaquing agent, gelling agent, aroma, perfume, sunscreen or colorant.

20. A method substantially as hereinbefore described with reference to any one of the Examples.

21. A dispersion of niosomes comprising a lipid lamella, or two or more substantially concentric lipid lamellae, encapsulating an aqueous phase E, the niosomes being dispersed in an aqueous medium D, obtained by a method as defined in any one of the preceding claims.

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